

The University of Chicago

**NORMAL DEVELOPMENT OF THE
PROSTATE AND SEMINAL VESICLES OF THE
RAT WITH A STUDY OF EXPERIMENTAL
POST-NATAL MODIFICATIONS**

**A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY**

**DEPARTMENT OF ZOÖLOGY
1935**

**By
DOROTHY PRICE**

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NORMAL DEVELOPMENT OF THE PROSTATE AND SEMINAL VESICLES OF THE RAT WITH A STUDY OF EXPERIMENTAL POST- NATAL MODIFICATIONS¹

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SEVEN TEXT FIGURES AND ONE PLATE (SEVEN FIGURES)

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INTRODUCTION

Numerous studies have shown that the functional state of the prostate and seminal vesicles of adult rats is dependent on male hormone. Gross size and secretory activity are conditioned, and regression is rapid after castration. The effects of castration and of subsequent rebuilding by hormone injections have been thoroughly studied in the adult by

¹ This investigation has been aided by a grant from the Rockefeller Foundation to The University of Chicago. The problem was suggested by Prof. Carl R. Moore to whom I am most grateful for unfailing encouragement and advice. I am indebted to those who kindly provided hormone preparations: Prof. F. C. Koch and Dr. T. F. Gallagher of the department of physiological chemistry and pharmacology, The University of Chicago, for bull-testis extract; Prof. H. B. Van Dyke and Mrs. Zonja Wallen-Lawrence of the same department for urinary hebin; Prof. R. G. Gustafson of the University of Denver for oestrin from human placentae.

many workers, and the glands have been used not only as qualitative indicators of hormone presence but also as quantitative indicators.

That the adult glands depend upon the presence of male hormone to preserve their normal size and secretory state is unquestionable. The problem then arises as to the relation of their origin, growth, and early differentiation to male hormone. It has sometimes been assumed that when castration is performed early in the life of the animal the accessory reproductive glands are immediately arrested and develop no further. This would indicate that growth and differentiation relatively late in prepuberal life are entirely conditioned by the presence of the testicles.

The degree of dependence of these developing secondary sex accessories upon the presence of male hormone and the related problems of the capacity of reaction of the young tissues have never been thoroughly analyzed in the rat. Thus it seemed of value to pursue the prostate and seminal vesicle studies and: 1) follow the normal development of these glands from their earliest anlagen to puberty; 2) perform castrations at a very early age and determine the effect of testis removal on the growth and differentiation of the glands; 3) administer male and female hormones and hypophyseal material to observe the ability of the very young tissues to respond to such treatments.

Much work has been done upon the response of immature testes and accessories to sex hormones and to gonad stimulating hormones from hypophyseal and pregnancy sources but in general, the treatments have covered a period relatively late in prepuberal life. For that reason, the experiments reported here were begun in most cases on animals within the first week of life (2 to 6 days). Some of the results of such experiments proved to be interesting and rather unexpected and thus offer new material for the analysis of the factors governing the growth and differentiation of the prostate and seminal vesicle glands.

MATERIAL AND METHODS

For this research, albino rats were used, from a stock which has been maintained in this laboratory for about 15 years. Approximately 350 rats were studied, of which 37 were foetal or newborn animals studied in serial section, 160 were older normals, 100 were castrates, and approximately 50 were normals or castrates given hormone treatments. Sixteen of the embryos were females which were examined to ascertain the development of the homologues of the male accessory glands.

For accurate study of the foetal material the age of the embryo was computed in days and hours from the time of observed copulations. The length of the accessory glands and ducts was found by counting the sections and multiplying by their thickness. The serial section study covered stages from 11 days post coitum to 5 days after birth. In addition, the urogenital region of one 35-day-old male was sectioned serially.

The remainder of the animals were killed when they were between 5 and 100 days of age and samples of the seminal vesicles and ventral lobes of the prostate were preserved for histological study. At autopsy, seminal vesicles with the accompanying coagulating gland were measured and weighed. Recorded seminal vesicle cell heights represent the average of ten ocular micrometer measurements for each animal. Since cell heights differ somewhat depending upon the location of the epithelium in the gland, ten of the highest cells were chosen for measurement in each preparation to make the results more nearly comparable.

Only the ventral lobes of the prostate were considered in the research on animals after birth. No weights or measurements of the intact gland were made in the experiments to be reported here. Cell heights and acinous diameters were measured with an ocular micrometer. The prostate presents even greater variation in cell height than the seminal vesicles and here again ten of the highest cells (usually in peripheral acini) were measured and averaged.

Tissues were fixed in Bouin's fluid as a routine and were stained in Harris' or Ehrlich's haematoxylin (Moore, Price and Gallagher, '30; Moore, Hughes and Gallagher, '30). Embryos were sectioned serially at 10 μ , testes at 7 and prostates and seminal vesicles at 4 μ .

Castration was performed through an abdominal incision and ether anesthesia was used. This method was entirely satisfactory for rats 2 days after birth and mortality in the young from all causes combined was less than 20%.

Injections in every case were given subcutaneously with one injection daily for oil extracts (male hormone) and two daily for aqueous extracts (phyone and hebin). Hypophysis implantations were made daily and the material injected deep into the leg muscles of the rats.

STUDY OF NORMAL DEVELOPMENT

Embryonic development

The embryological development of many parts of the reproductive system of the rat and mouse has been studied by various workers. The main attention of the present study has been centered upon the development of the seminal vesicles and prostate. However, the development of the wolffian and müllerian duct systems in the two sexes has been reexamined and the new points of interest will be reported elsewhere.

In the rat the sexes can first be recognized by a study of the histological structure of the gonads at 14½ days post coitum. Although the gonads begin their differentiation relatively early in embryonic life the anlagen of the accessory reproductive glands appear relatively late.

Development of the prostate in the male from the anlage stage to 5 days after birth

The anlage of the prostate in the male rat can first be seen in the 19½-day embryo. The prostatic anlagen extend from the urogenital sinus as several small solid cords of cells. Their arrangement is roughly bilaterally symmetrical in relation to the dorso-ventral mid-line of the sinus (fig. 1).

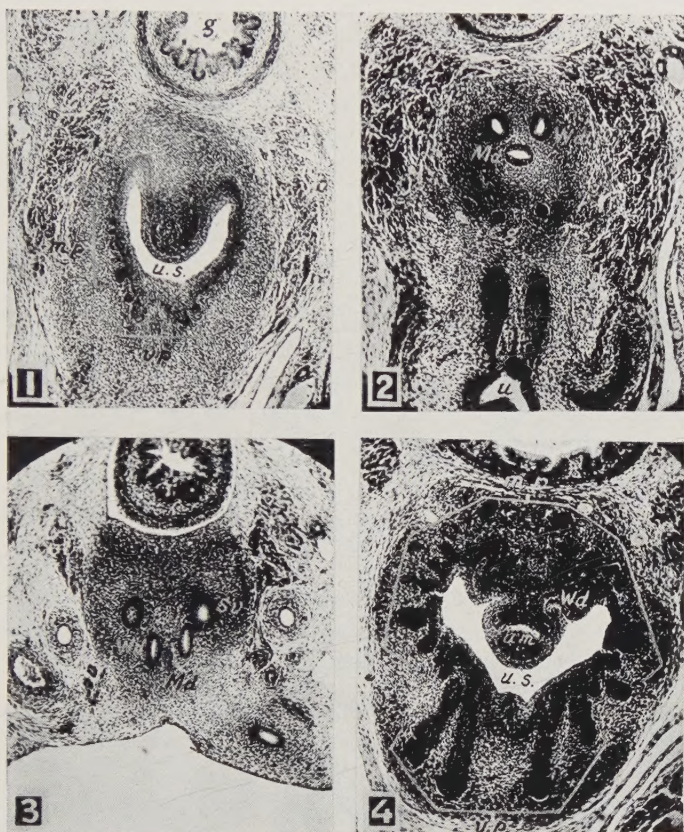


Fig.1 Ventral and mid-lobes of the prostate of a 19-day and 12-hour male embryo.

Fig.2 Dorsal cranial prostate (coagulating gland) in a more cranial section in same embryo as figure 1.

Fig.3 Seminal vesicle anlage in an 18-day and 18-hour male embryo.

Fig.4 Prostatic anlagen in a 21-day and 12-hour male embryo.

c.g., coagulating gland

m.p., mid-prostate

v.p., ventral prostate

m.d., müllerian ducts

w.d., wolffian ducts

u.m., uterus masculinus

u.s., urogenital sinus

s.v., seminal vesicle

g., gut

Although the anlagen are not definitely divided into lobes the coagulating gland can be recognized as a pair of solid cords that extend cranially into the genital cord ventral to the müllerian and wolffian ducts (fig. 2). In later development they come to lie close to the seminal vesicles to which they are bound. The anlagen of the coagulating gland join the sinus cranial to the entrance of the wolffian ducts and müllerian ducts. The mid-lobe of the prostate is represented by the buds of cells that extend laterally from the sinus (fig. 1), and the posterior lobe by the four or five more prominent cords of cells that grow ventrally from the sinus on each side of the mid-line.

The length of the sinus involved in gland formation increases as embryonic life proceeds and the buds increase in number and length (fig. 4). The dorsal side of the sinus gives off many anlagen caudal to the point where the wolffian ducts and müllerian ducts open on the sides of the colliculus seminalis. These are primordia of the mid-lobe. The coagulating gland anlagen join the paired groove-like extensions from the prostatic sulci which extend dorsally from the base of the colliculus. Thus they always join the sinus cranial to the other genital ducts and gland ducts.

The acini grow in length, coil and branch but do not acquire lumina in embryonic life. The cords of cells that form the ducts and acini of the ventral lobes bend caudally as they grow while those of the mid-lobe grow laterally and cranially. At birth the acini of the three prostatic lobes are still solid cords of cells that have grown out some distance on all sides of the sinus. There are no sharp divisions into lobes but all three pairs can be recognized.

Between birth and 5 days, growth continues and the ducts develop lumina (fig. 5). Now it is evident that the ventral lobes have three to five ducts each, the mid-lobes many ducts, and the anterior lobes one main duct each. The ducts of the coagulating gland join the deep grooves around the base of the colliculus cranial to the opening of the ejaculatory ducts. Some of the ducts of the mid-lobes open into the above-mentioned grooves and some into the dorsal and lateral parts

of the sinus proper, while one pair runs caudally and opens dorsally at the point where the uterus masculinus appears in the colliculus. The ducts of the ventral lobes join the



Fig. 5 Section through the prostate region in a male 5 days after birth.

d.c.g., duct of coagulating gland
m.p., mid-prostate
d.v.p., duct of ventral prostate
u., urethra

v., vas deferens
a., ampulla of vas deferens
s.v., seminal vesicle
g., gut

ventral surface of the sinus caudal to the ejaculatory ducts but cranial to the longest pair of ducts of the mid-lobes.²

The three paired lobes are still not clearly demarked. The right and left ventral lobes are separated by a narrow connective tissue boundary, and the right and left coagulating glands are, of course, distinguishable. However, the acini of the mid-lobes and ventral lobes adjoin and it is not possible to tell them apart except by position. In addition, the mid-lobe acini cannot be separated in their dorsal extension from coagulating gland acini which have branched from the main ducts. All of the acini are embedded in a connective tissue stroma.

Since much of this study has been devoted to the castration of animals in the first week of life, this 5-day-stage may be chosen as typical of the state of the animal at the time of castration. The acini, as can be seen in figure 5, are solid. This is true in the distal part of all the lobes although the ducts and perhaps a very small region in the adjoining proximal acini have lumina. The ducts have a simple epithelium with somewhat crowded nuclei. The acini are coiled and branched and about 30 μ in diameter although they vary. Their cytoplasm is loose, open and homogeneous and the nuclei are small and somewhat lighter staining than the nuclei in the adult gland but they possess the characteristic chromatin. The cells rest upon a definite basement membrane as in the adult. Mitoses are very numerous and conspicuous.

² Stutzmann (1898) reports that in *Mus decumanus* the ventral lobes have a single long well-developed duct for each lobe but in his figure the primordia of the dorsal cranial prostate (or coagulating gland) which are obviously dorsal to the sinus are labelled "the free lying or ventral lobes" (fig. 4). Rauther ('03) has followed him with the same observations for the ducts of the ventral lobes, as has Disselhorst ('04). Our findings do not corroborate this for there are at least three ducts from each ventral lobe. The anterior lobes or coagulating glands are each drained by one main duct. This was also observed by Pallin ('01) although Rauther observed three. In all of Stutzmann's figures except two (figs. 1 and 2) the labels of the 'festliegend' or dorsal and 'freiliegend' or ventral lobes are reversed. There is general agreement that the mid-lobes open by many ducts.

Development of the prostatic homologue in the female from the anlage stage to 5 days after birth

The female homologue of the prostate gland of the male is the para-urethral glands of Skene. Pallin ('01) calls the glands of Skene of the human female homologous to portions of the ventral and dorsal male prostate. Marx ('32) states that in the rat they correspond to the median lobe or the coagulating gland.

They arise in the foetal female rat of 19 days and 19 hours as small single or paired swellings on the urethral wall. The female glands originate at a point in the urethra that is cranial to the place where the walls of the utero-vaginal canal and the urethra fuse to make what was in younger embryos a well-developed male type of urogenital sinus, but now in the female is solid in the dorsal or genital portion.

Their further development up to 5 days after birth consists of growth and slight coiling but they do not branch nor develop lumina. Their growth is neither consistent nor great. Marx reports that a 'prostate' is found now and then in the adult female of the white rat.

Development of the seminal vesicle in the male from the anlage stage to 5 days after birth

The first gland primordium to be found in the developing male rat is that of the seminal vesicle which begins its development when the foetus is 18 days and 18 hours of age. Its form is that of a simple diverticulum which grows dorsally and cranially from each wolffian duct (fig. 3). These paired diverticula continue to lengthen until at 21½ days the cranial tips turn back ventrally to form the flexure characteristic of the adult gland. The lumen of each gland remains unbranched up to birth so that up to that time the seminal vesicles are simple sac-like organs. The epithelium is like that of the wolffian ducts with tall columnar cells crowded into pseudo-stratification and with many mitotic figures evident.

The opening of the seminal vesicles into the wolffian ducts grows progressively caudad but in the 5-day-old rat true ejaculatory ducts are still present and serial sections of a 35-day-old male revealed that there is a short ejaculatory duct at that age. In the adult, probing showed that a short ejaculatory duct may persist.

Within the first 5 days after birth the central lumen of the seminal vesicle begins to develop small side branches (fig. 5). Both the central lumen and the side branches are surrounded by a fibro-muscular sheath and are embedded in abundant connective tissue. The epithelium is simple although it frequently presents a crowded pseudo-stratified appearance. The cells are tall columnar with basal nuclei which have several dark staining nucleoli. The cytoplasm is fibrillar or finely granular and the basement membrane is frequently not clearly distinguishable.

The homologue of the seminal vesicle does not appear in the female rat. This may be due to the fact that the wolffian ducts in the female begin their degeneration before the time of appearance of the accessory reproductive glands and are missing or incomplete in that section which would form seminal vesicle anlage.

Later growth and differentiation

Prostate. The early growth and development of the three lobes of the prostate from the anlage stage to 5 days after birth have been described. Only the ventral lobes were studied after birth. It will be recalled that at 5 days after birth the acini of the ventral prostate are solid except for small areas adjacent to the ducts. The ducts themselves possess lumina and have a smaller diameter and relatively low epithelium. The further development of this part of the gland can be understood best by descriptions of definite stages that show advancement. Growth in gross size, however, involving increase in acinous length and in branching proceeds steadily.

Ten days. At 10 days after birth some of the acini in the periphery of the gland have begun to show small lumina. The process of hollowing out of the acini seems to be progressive and proceeds from proximal to distal in the gland. The acini vary in size but develop lumina when they are approximately 35μ in diameter. In the larger solid cords the nuclei show a tendency to become arranged around the periphery of the cord. When lumina develop, the cells can then be measured and are usually about 17μ high. Their cytoplasm is somewhat light and fibrillar and the nuclei are basal.

Morphogenesis is a complicated process in the gland at this time because simultaneously the cells are increasing in number (mitoses are numerous) and in height, and the lumina are enlarging rapidly. It is well to repeat here that measurements were made of the highest available epithelium.

Twelve days. At this stage more of the acini are hollow and a new structure has appeared, for in a few cells in some animals light areas are visible about halfway between the nucleus and the lumen end of the cell. These are pale but have the same appearance and occupy the same position as those that have been previously described for the adult prostate (Moore, Price and Gallagher, '30). They were found to be present invariably in adult functional glands and formed a striking light band in the epithelium. Their presence has been used to denote secretory activity of prostate cells. These pale light areas in the prostate of the 12-day-old rat occurred in three out of six animals examined at this age. The cytoplasm around the light areas is also pale and finely granular or fibrillar. The acini are developing small villi that extend into the lumina. These projections are instrumental in the branching of the alveoli for they fuse with the opposite wall of the lumen and thus cut off side branches. This is much more conspicuous in older animals where the villi are more numerous (fig. 9).

Fifteen days. Thirteen animals were examined at this age and all possessed the diagnostic light areas. In some animals they are so consistently present that they give almost

the appearance of those of the adult gland except for the fact that they are not so strongly marked as in the adult and the acini are not so distended. There are still a few solid acini but the majority have lumina and many are relatively distended as compared with the acini of the 12-day-old rat. Figure 7 shows a typical 15-day-old gland with high epithelium and light areas that are not so conspicuous as in the adult but are unmistakable. The light areas are always present in animals of 15 days of age or over and they grow progressively stronger and more marked.

Twenty-five days. By 25 days the histological structure of the prostate approximates that of the adult. A typical 25-day stage is shown in figure 9. This differs from the adult only in smaller acinous diameters and lower epithelium.

As the gland grows and acini become more distended, accurate measurements become more difficult. Table 1 gives the measurements for cell heights and acinous diameters. The contours are irregular in the largest alveoli and consequently measurements were taken of acini that were nearly round in cross section. For that reason the largest acini could not usually be included. Graph A shows that the acini increase steadily in diameter but that the increase is much more rapid for the first 30 to 40 days. Growth continues, however, and villi increase. Maximum cell height is practically realized within the 30-to 40-day period although there is considerable variation. The early acquisition of cell heights that are very close to maximum in the prostate bears out the histological findings of early differentiation. Mitoses are most numerous in young animals up to 20 days of age. They are fairly numerous up to 35 days and are rare after that although they have been observed occasionally up to the end of the 100-day period and in adults.

No weights or measurements were made on the ventral prostate. It does not show a sudden increase in gross size at 'sexual maturity' comparable to that of the seminal vesicle.

In the prostate, then, we have an accessory reproductive gland which reaches the histological structure of that of a

normal adult before the rat is 3 weeks of age. The seminal vesicle does not reach a comparable stage of development until the rat is at least 5 weeks old. Secretory differentiation

TABLE 1

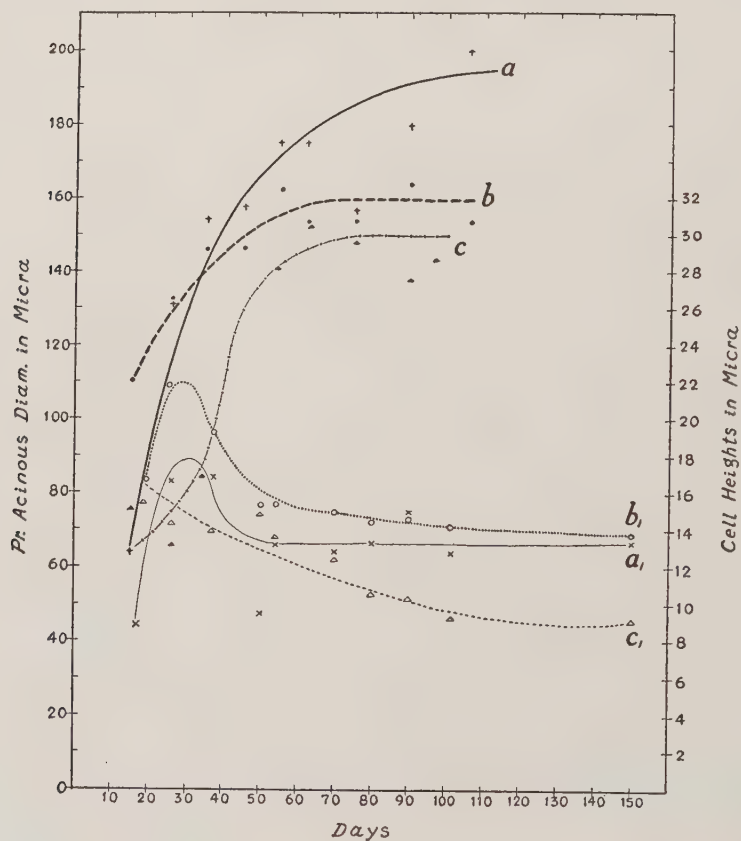
Seminal vesicle cell heights, prostate cell heights and acinous diameters in normal and castrate rats (castrated at 2 to 6 days of age)

AGE, DAYS	NUMBER OF CASES		PROSTATE						SEMINAL VESICLE			
			Average cell height, μ		Average acinous diameter, μ		Light areas		Average cell height, μ		Sec. Gr.	
	N	C	N	C	N	C	N	C	N	C	N	C
11	2	1	18.2		42.7	30.1	—	—	15.4	14.0	—	—
12	6	8	18.9		46.9	35.9	3+, 3—	—	15.8	15.4	—	—
15	9	8	21.7	16.1	64.0	39.8	+	—	16.9	15.4	—	—
16	2	1	24.5	16.1	66.0	35.0	+	—	15.4	16.1	—	—
17	1	1	24.5	18.9	77.7	49.0	+	+	12.6	14.0	—	—
18	4	2	25.9	17.5	81.9	62.3	+	1+, 1—	14.1	15.4	—	—
21	5	1	24.5	21.7	95.9	65.8	+	+	13.8	15.4	—	—
22	3	2	23.8	16.8	91.0	42.7	+	1+, 1—	13.0	14.7	—	—
25	4	1	28.0	22.4	103.0	73.5	+	+	12.7	14.0	—	—
26	1	1	28.7	22.1	112.0	84.0	+	+	13.3	12.6	—	—
27	1	2	29.4	22.1	112.7	92.4	+	+	11.9	14.0	—	—
28	1	2	28.0	22.4	122.5	102.2	+	+	11.9	14.7	—	—
30	8	3	26.6	23.8	144.0	98.0	+	+	14.7	14.0	—	—
34	1	1	30.1	21.7	155.0	85.4	+	+	14.0	12.6	—	—
35	4	3	30.1	21.7	139.0	89.6	+	+	15.0	13.3	—	—
36	6	1	30.1	14.0	152.0	74.2	+	—	19.6	14.0	+	—
40	1	6	31.5	18.2	162.0	81.2	+	4?+, 2—	21.7	14.0	+	—
52	1	2	31.5	14.0	197.0	68.6	+	—	29.4	13.3	+	—
55	1	2	33.6	16.1	170.0	60.9	+	—	25.9	14.0	+	—
70	1	2	30.8	14.7	157.0	63.7	+	—	28.7	11.9	+	—
80	1	2	37.1	14.0	180.0	65.8	+	—	25.9	10.5	+	—
90	1	2	29.4	14.7	180.0	75.6	+	—	29.4	10.1	+	—
101	1	3	30.8	14.0	200.0	63.9	+	—	28.7	9.3	+	—
150	2	5	32.2	13.7	200.0	67.4	+	—	30.1	9.1	+	—
Total	67	62										

N, Normal; C, castrate.

of the prostate gland is attained long before so-called 'sexual maturity' is reached, as judged by the presence of spermatozoa in the testes and secretion granules in the seminal vesicle. The last two appear together at about 35 days.

It seems necessary to include here a discussion of the classification of the three lobes of the prostate and their histology. The original classification of the lobes by Müller as quoted by Stutzmann (1898) was 'glandulae prostaticae anteriores,' 'mediae' and 'posteriores' and in his terminology 'anteriores' referred to the free ventral lobe, 'mediae' to the middle lobe and 'posteriores' to the coagulating gland. Pallin



Graph A Growth curves of seminal vesicle cell heights and prostate cell heights and acinous diameters in normal and castrated rats. The data for ninety-three normals and sixty-three castrates have been grouped into 10-day periods. a, normal prostate acinous diameter; a', castrate prostate acinous diameter; b, normal prostate cell height; b', castrate prostate cell height; c, normal seminal vesicle height; c', castrate seminal vesicle cell height.

('01) used the terms 'dorsal cranial' (coagulating gland in later classification), 'dorsal caudal' (middle lobe) and 'ventral' which seems from this study to be the most convenient usage. Rauther ('03) used Müller's classification but says that while the middle lobes resemble the ventral lobes in gross appearance, their histology and the homogeneous character of their secretion are quite similar to that of the lobes that are attached to the seminal vesicles, and Disselhorst ('04) and Rietschel ('29) agree with this. Stutzmann could find no macroscopic division between the middle lobes (mediae) and the dorsal cranial lobes (posteriores) so preferred to consider the prostate of *Mus decumanus* as consisting of only two pairs of lobes.

Walker in 1910 described a special function for the dorsal cranial lobes that are bound to the seminal vesicles, and because of this function he called them 'coagulating glands.' He described the histology of these lobes as different from that of the 'anterior' (ventral) and posterior (middle) lobes. These last two lobes he considered identical.

In an earlier paper (Moore, Price and Gallagher, '30) it was stated that the middle and posterior lobes had the same histology. This statement must be somewhat modified in the light of further work. The middle lobe resembles the distended portion of the coagulating gland in regard to the shape and folding of the acini but the epithelium itself differs from that of both other lobes.

Seminal vesicle. At 5 days after birth the seminal vesicles can be dissected for weighing. The weight of both together averages about 0.9 mg. The histological structure, as has been stated in a preceding section, is very simple with small side branches just beginning to grow out from the central lumen (fig. 5). The development of the seminal vesicle differs from that of the ventral prostate and the stages that need be described are very few and are chosen somewhat arbitrarily.

Fifteen days. At this age the central lumen which runs the length of the gland has given off lateral growths which are

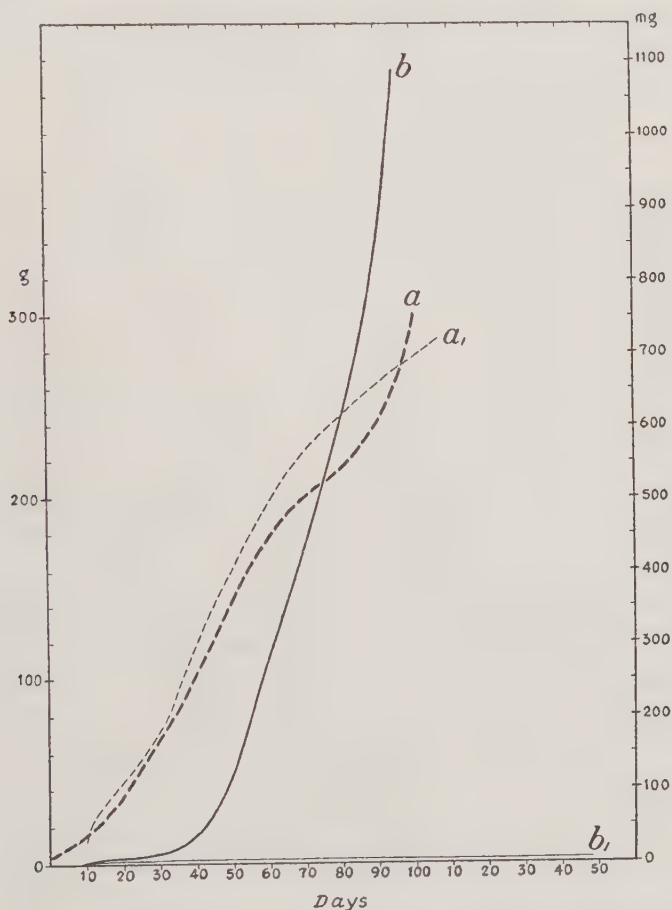
ramified and very complex so that the epithelium in the side branches is folded. Many mitotic figures in the epithelium give evidence of the rapid growth of the side branches and in recesses the epithelium is consistently higher than in the central lumen. The cytoplasm is finely granular as in the younger stages. The weight of the glands at 15 days is about seven times that of the 5-day-old glands.

Thirty days. At this stage the gland structure has become increasingly complex thus giving larger and larger amounts of epithelial surface, but the cells themselves average less than $15\ \mu$ in height. The cytoplasm has always been granular but this becomes more marked now and large protoplasmic granules are conspicuous. It is not definitely known whether these are 'pre-secretion' granules or whether they are unrelated to secretion products. None of these granules possessed halos and so could not be classified as typical secretion granules.

Cell divisions have been numerous from early embryonic life to about 20 to 25 days after birth but they are now less frequent although they have been found up to 76 days. While they were not observed later they are undoubtedly present in small numbers.

Thirty-six days. This marks a definite stage in the development of the seminal vesicles for true secretion granules surrounded by pale halos appear in the cytoplasm. They are not so large nor so numerous as in older animals but they are definitely present at this and all subsequent ages in normal animals. The cell height now has increased to about $20\ \mu$ and the weight of the gland shows the beginning of a rapid rise which is still more marked later. Graph B gives a curve of weight increase for the seminal vesicles of 100 normal animals. It is evident that weight begins to increase decidedly between 30 and 40 days and the slope is very steep from then to the end of the observed period. This weight increase represents both growth, and storage of secretion for it is the accumulation of secretion that accounts for a large part of the weight of the gland and causes some of the rapid

rise in weight. The glands continue to grow beyond the 100-day period and in older and heavier adults the weight is frequently $1\frac{1}{2}$ to 2 gm. The weight varies greatly however and in many cases does not seem to be closely correlated with body weight or testis size.



Graph B Growth curves of body weights and seminal vesicle weights in normal and castrated rats. The curves were drawn by inspection through the scatter of points for 160 normals and sixty castrates for body weight, and 100 normals and sixty castrates for seminal vesicle weight. Body weights are given in grams and seminal vesicle weights in milligrams. *a*, normal body weight; *a'*, castrate body weight; *b*, normal seminal vesicle weight; *b'*, castrate seminal vesicle weight.

Graph A gives the curve of normal increase in cell height and this shows clearly a long delay and a rapid increase. Curves for both weight and cell height follow the curve of growth typical for reproductive tissue as given by Harris, Jackson, Patterson and Scammon ('30). The growth of prostate cell heights and acinous diameters is not in agreement with this type of curve. It is possible that if weights of the gland had been taken they might have been found to increase in some such way. Callow and Deanesly ('35) in a study of prostate and seminal vesicle growth in rats of 42 gm. to 250 gm. show typical curves for reproductive tissue for the weight increase in both glands but there is an initial lag in the seminal vesicle as compared with the prostate. Neither gland enlarged appreciably below a body weight of 100 gm.

This study of the seminal vesicle of the rat leads to the conclusion that the gland grows slowly and shows no differentiation of the epithelium until after 30 days when differentiation occurs and increase in cell height and gland weight begin to be marked. Secretion granules are present in seminal vesicles of animals 36 days of age or over. Before that time none with halos have been found although distinct protoplasmic granules are present. This behavior is exactly the opposite of that of the prostate in which differentiation as judged by the presence of typical light areas in the cells begins at 2 weeks of age, and increase in cell height and acinous diameter go on rapidly without the long delay characteristic of the seminal vesicle growth and cell height.

EXPERIMENTAL METHODS OF MODIFYING THE GLANDS

Castration

Castrations were successfully performed on sixty-five males within the first week of life (2 to 6 days) and on thirty-five others from 10 days of age to adults. Castration effects were studied on seminal vesicle sizes, weights, and histology and on prostate histology. This study included the measurement of seminal vesicle cell heights, prostate cell heights and acinous

diameters following castration. The litter has been used as a unit in all experiments. The body weights of 160 normals and 60 castrates (operated 2 to 6 days) have been plotted in graph B. The growth curves of normal animals and early castrates show no great difference during the 100-day period although the slope of the curves at the end of the period is suggestive. However, the experimental period was not long enough to show the castrate loss of weight reported by van Wagenen ('28) and Commins ('32).

Effects of castration on the prostate gland

The effects of postpuberal castration on the prostate have been reported in detail elsewhere (Moore, Price and Gallagher, '30) and it may be recalled that after castration a number of changes take place: The characteristic light areas disappear from the cells within 4 days; there is a decrease in cell height and acinous diameter and an apparent increase in the connective tissue stroma. The cytoplasm becomes pale and vacuolated, and the nuclei somewhat smaller and pyknotic. In addition, the gland decreases in gross size and changes from firm pink lobes to flabby brownish tissue.

It seemed very probable that in rats castrated a few days after birth the prostate gland might cease to grow and differentiate. Since the normal prostate shows light areas as early as 12 days, castrations were done before and after this stage.

Sixty-five rats were castrated between 2 and 6 days of age and sacrificed between 11 and 150 days of age. Table 2 gives the results found in four of the fourteen litters included in these experiments and will suffice to make clear the effects of early castration.

In litter 1, for example, three rats were castrated at 4 days of age and killed with two littermate controls at 15 days of age. The approximate state of the prostate at the time of castration is shown in figure 5 (a rat at 5 days of age), and the appearance of the castrate at autopsy is given in figure 6. In figure 7 it can be seen that the prostate of a normal litter-

TABLE 2
A comparison of control and castrate littermates (castrated 2 to 6 days)

LITTER	RAT	TREATMENT	AUTOPSY		SEMINAL VESICLE				PROSTATE		
			Age	Weight	Size	Weight	Cell height	LA	Cell height	Acinous diameter	
			days	gm.	mm.	gm.	μ		μ	μ	
1	908	Normal control	15	36	3.8×2.1	0.0065	17.5	+	22.4	66.5	
	909	Normal control	15	38	3.0×2.2	0.0064	19.6	+	22.4	67.9	
	906	Castrated at 4 days	15	34	2.5×1.5	0.0034	16.1	—	15.7	44.8	
	905	Castrated at 4 days	15	33	2.5×1.5	0.0024	16.1	—	17.5	37.8	
	907	Castrated at 4 days	15	33	2.5×1.6	0.0025	14.0	—	14.7	34.6	
2	863	Normal control	17	48	3.8×2.1	0.0078	12.6	+	24.5	77.7	
	862	Castrated at 3 days	17	48	2.5×1.3	0.0027	14.0	+	18.9	49.3	
	864	Castrated at 3 days	34	131	3.9×1.6	0.0042	12.6	+	21.7	85.4	
3	358	Castrated at 2 days	12	20	1.7×0.5		16.8	—	...	37.4	
	359	Castrated at 2 days	15	31	2.5×1.1		17.5	—	17.5	37.1	
	360	Castrated at 2 days	18	34	3.2×1.1		14.7	—	16.8	49.0	
	361	Castrated at 2 days	26	55	3.0×1.3		12.6	+	24.1	84.0	
	362	Castrated at 2 days	30	70	3.0×1.1		15.4	+	24.5	107.1	
	363	Castrated at 2 days	36	116	3.2×0.7		14.0	—	14.0	74.2	
4	549	Normal control	16	27	4.2×1.6	0.0054	15.4	+	25.9	60.2	
	548	Castrated at 6 days	16	29	2.5×1.0	0.0020	16.1	—	16.1	35.0	
	852	Normal control	27	64	4.1×3.0	0.0123	11.9	+	29.4	112.7	
	850	Castrated at 6 days	27	59	2.6×1.4	0.0016	13.3	+	22.4	90.3	
	851	Castrated at 6 days	27	62	3.1×1.3	0.0022	14.7	+	23.8	94.5	
	854	Normal control	35	70	5.0×1.9	0.0106	13.3	+	30.1	130.0	
	853	Castrated at 6 days	35	90	3.3×1.5	0.0032	14.7	+	24.5	102.9	

mate control now possesses pale light areas and moderately distended acini.

The prostate of the castrate was smaller in gross size than its control but was large enough to be easily recoverable at autopsy which means that it had greatly increased over the 4-day size and mitoses were seen in the histological preparation. While much below the normal in differentiation, cell height and alveolar diameters, it proved to be further developed than the stage at which castration was performed. In figure 6 it can be seen that small lumina are present in the acini of the castrate. These begin their development in the castrate by 12 days of age as is shown in eight animals killed at that age but not listed in table 2.

There is considerable variation in the diameters of acini in normal animals and the variation is even greater among castrates as is illustrated by the measurements for rats 905, 906 and 907. Cell heights also varied but were found to be more consistent than acinous diameters as would be expected since the highest epithelium was selected for measurement while the acinous diameters were taken at random.

Thus the castrate prostate increases in gross size and acinous diameter and forms lumina in the solid acini. It also proceeds in differentiation to form light cell areas as shown in litter 2 in which two animals were castrated at 3 days of age and one killed with the normal littermate at 17 days of age, the other at 34 days of age. The 17-day-old castrate prostate acini not only have lumina and are more distended than in the younger castrates but some of the cells of the acini have very pale light areas of the type usually found at about 12 days in the normal animal. These light areas are not nearly so consistent and strong as in the normal control, and epithelial height and acinous distention are below the normal. However, the castrate prostate here shows not only growth but differentiation. The littermate castrate killed at 34 days shows a greater distention of acini and high epithelium in which a few pale light areas were found. Differentiation was evident in the 17-day-old castrate and was maintained up to 34 days in a castrate littermate.

It is interesting to follow through the progressive development and regression in one litter. In litter 3, six animals were castrated at 2 days of age; one each was killed at 12, 15, 18, 26, 30 and 36 days. The 12-day-old animal has solid acini in the prostate; at 15 days lumina appear in the acini and the acini have increased in size; at 18 days, further acinous growth is evident although no light areas are visible. At 26 days, well-developed light areas are present in a high epithelium and the alveoli have increased in size. At 30 days, there are consistent strong light areas and still larger acini in which there is abundant secretion. In great contrast is the littermate killed at 36 days, for the histological picture is that of a castrate with low epithelium, smaller acini and increase in interacinous connective tissue. This one litter can serve to illustrate the whole process of early development, differentiation and ultimate regression. Litter 4 substantiates these results.

Examination of the data from these and from other unreported litters shows that differentiation (in the form of light areas) takes place when the castrate is approximately 17 days of age and further increase in strength of light areas, cell height and acinous diameter continues up to about 30 days after which regression sets in. The light areas then disappear, the epithelium becomes lower and the acini decrease in size. At 40 days only a few very questionable light areas have been observed (see table 1). The castrates never reach the degree of development of their littermate controls but always have smaller acini, lower epithelium and weaker light areas. In some castrates, high epithelium and light areas are limited to a few acini. The gross size of the castrate prostate is smaller than in normal littermates but up to 40 days the lobes are fairly large, pink and firm. After that they become flabby and resemble the typical adult castrate.

In table 1 the data are given for some of the animals operated in the first week of life. The prostate cell height and acinous diameter can be seen to increase up to 30 days and to decrease after that. The light areas occur in sixteen

out of eighteen cases between the ages of 17 and 35 days, and four animals at 40 days have faint indications of light areas. Graph A shows the curves of normal and castrate cell heights and acinous diameters. It is evident that the prostate growth as judged by cell height and alveolar distention continues for at least 3 weeks after castration and that this growth while well marked is not so great as in normal controls.

Figures 8, 10 and 11 illustrate the castrate prostate in animals of 25, 30 and 52 days of age. Figure 8 must be compared with figure 9, the normal littermate at 25 days, and figure 11 with figure 12 which is an adult animal castrated for 21 days. In the castrate at 25 days of age the epithelium is high and the light areas are readily distinguishable. At 30 days there is some high epithelium with light areas and much low epithelium in the same gland (such as is shown in the acini at the bottom of the figure). At 52 days the gland is castrate in type but is not nearly so conspicuously regressed as the 20-day adult castrate although the 52-day animal has been castrated for 50 days.

Since differentiation of the prostate to a secretory state occurs in spite of castration at 2 days of age, it was desirable to determine whether castration subsequent to the appearance of the light areas (on day 12) would permit this state to be maintained.

Nine litters composed of twenty-four males castrated between ages 16 and 48 days and sacrificed after 10 to 47 days of castration, and fifteen controls were used to test the point. One litter only will be cited in detail since it combines results of castrations performed before and after the prostate light areas appear. In this litter of six males two animals were castrated at 5 days of age and two at 16 days of age. One control was killed at 17 days and one at 28 days when all four castrates were sacrificed. The interesting point is that the two rats castrated at 5 days and the two castrated at 16 days all have light areas, high epithelium and moderate distention at 28 days. In the former the light areas have developed, while in the latter, they have been preserved for 12

days (the 16-day control had them) and the acini have increased in size over the 16-day control.

The remaining litters demonstrate further that in all animals castrated at 21 days of age or over and killed more than 20 days later, regression and atrophy have taken place in the prostate. This atrophy reaches a plateau in respect to epithelial height and acinous diameter in 20 to 30 days. This does not mean that there is no change in the regressing gland after that time. On the contrary, the prostates grow somewhat smaller with increasing age, the connective tissue increases, the cytoplasm becomes more vacuolated and the nuclei smaller and more pyknotic. In these older animals pyknosis begins in the nuclei of the lower strata after about 20 days of castration, but there are always many normal nuclei to be found even after long periods of castration. The spotted and dark staining nuclei are never as striking in the prostate as in the castrate seminal vesicle.

It is a surprising fact that light areas can be found after 30 days of castration when the operation is done on rats within the first week of life; for 10 to 20 days if castrations are performed between 16 and 21 days of age; and for only 4 days if castrations are done on adults. After 20 or 25 days of castration in an adult the prostate has reached a stage of atrophy shown in figure 12. After the same length of castration (done in the first week of life) the young castrate prostate is nearly normal as figure 8 shows. However, after 50 days of castration the young prostate in figure 11 is typically castrate and is comparable to the adult castrate in figure 12.

In order to determine how comparable were prostates of prepuberal and postpuberal castrates, five young at 2 days of age and an equal number of adults were castrated, both groups being killed 148 days after operation. At autopsy, the prostates of the prepuberal castrates were much smaller than those of the adult castrates but histological study shows no marked differences between the two groups as a whole. Epithelial height is similar but the acini of the older castrates are in general less distended. Qualitative differences are not

apparent between the pre- and postpuberal castrates. There is an overlap in prostate histology in the two groups and any differences are slight and quantitative. The gland of the prepuberal castrate is not a simple gland that has never undergone development, nor in this regard, does it resemble that of any young normal animals. As has been shown it is a gland that has developed after castration, and subsequent involution has followed so that its resemblance to the adult castrate would be expected.

Effects of castration on the seminal vesicles

Moore, Hughes and Gallagher ('30) described the normal histology and cytology of the adult seminal vesicle of the rat and the effects of postpuberal castration and subsequent repair by testis hormone injection. In brief, castration effects include the loss of secretion granules within 48 hours, reduction of cell height, and degenerative nuclear changes which include pyknosis and decrease in size. The gland undergoes a rapid decrease in size and weight. Observations reported here revealed a marked difference between the prostates and the seminal vesicles of young rats in respect to growth and differentiation.

In tables 1 and 2 are given some of the results of very early castrations. The seminal vesicles of a 5-day-old rat are shown in figure 5 and represent the approximate condition of the gland when the early operations were performed. The central lumen is beginning to give off simple side branches but the gland is very little more than a sac with the cranial end turned back in the flexure characteristic of the adult. The two seminal vesicles of 5-day-old rats weigh on the average approximately 0.0009 gm. It can be assumed that the glands in rats operated within the first 6 days of life weighed at operation little more than this, and frequently less, especially in the cases where operations were done at 2, 3 or 4 days.

It must be stressed that it is difficult to dissect accurately for weighing organs as small as the young castrate seminal vesicles and this source of error must be taken into account.

Litter 1 in table 2 illustrates some of the effects of early castration on the seminal vesicle. Three rats were castrated at 4 days of age and killed at 15 days of age with two controls. At the time of operation the glands in all probability weighed less than 0.0009 gm. Seminal vesicle weights of the castrates show an increase of approximately three times their probable operative size while those of the normals increased seven times.

Histological examination brings out that the castrate seminal vesicles show a widening of the central lumen and a further development of simple lateral branches. The normal glands at this age have a very complicated structure with side branches from which additional branching and folding have taken place. The castrate seminal vesicles have developed markedly from the 4-day state but are far behind the normals. Mitoses are numerous in both normals and castrates and the epithelium is somewhat lower in the castrates than in the normals.

The remaining litters on table 2 and the data on table 1 add only a few points. The seminal vesicles of the early castrates show a tendency to increase still further in weight after longer periods of castration but in only one case did the two vesicles weigh more than 6.6 mg., a weight reached by most normal animals at 15 days of age. Graph B gives the growth curve for castrate seminal vesicles as compared with the normal. It can be seen that there is a slight initial increase up to about 30 days but that the growth of the castrate is insignificant as compared with the normal.

The structure of the prepuberal castrate gland remains at any age as simple as in the 15-day-old castrate. The central lumen remains wide and there are very simple lateral branches but no further development. The epithelium is slightly higher in these very young castrates killed early than in those allowed to live longer periods as is shown in graph A. This is probably correlated with the slight additional growth which occurs. Mitoses have been seen in castrate seminal vesicles in animals up to 27 days of age and are probably

present later although they were not observed. Some pyknosis was observed at 30 days of age and a greater amount at 90 days of age yet many nuclei were normal.

The data for animals castrated at 15 days of age and later show that in animals castrated under 30 days of age some growth of the glands continues for a time. The seminal vesicles of the castrates are larger than those of the controls killed at the time of operation. This does not seem to be the case in rats castrated later than 30 days. In rats operated after further development of the glands, the histological structure is found to be more complicated than in the very early castrates.

In rats castrated at 2 days of age and as adults with sacrifice 148 days later, weights of the seminal vesicles of the young castrates averaged 6.5 mg. and those of the rats castrated as adults 47.9 mg. The histology of the seminal vesicles of prepuberal castrates reveals simple lumina and epithelium and pyknotic nuclei. Adult castrates have a much more complicated seminal vesicle structure as would be expected considering the stage of their development before regression. The epithelium is lower and the pyknosis of the nuclei very much more marked than in the prepuberal castrates. Comparison of the two groups shows that the prepuberal castrate seminal vesicles give the appearance of simple glands that have undergone little development, therefore little subsequent involution, although the nuclei show some regression. The postpuberal castrates have larger glands even after regression, as would be expected, but these were flabby and soft as in all adult castrates. They show greater involution in the way of markedly degenerative nuclei and very low epithelium.

In summary, the prostate and the seminal vesicles of prepuberal animals differ in their reaction to castration. After early castrations the prostate undergoes growth and differentiation up to 30 days of age after which gross size decreases and atrophy occurs. The resulting histological state of the gland is essentially similar to that of adult castrates. The castrate seminal vesicles on the other hand develop only

simple branched lumina. They grow slowly and to a limited extent up to 30 days of age (normally never exceeding the size of a 15-day-old normal) but remain simple and undifferentiated in structure. They show no regression in size but the epithelium decreases in height after long periods of castration and the nuclei undergo degenerative changes. These castration effects are not so marked as in the adult castrate. Neither gland is completely stopped in its growth by such early testis removal although the seminal vesicle does not show the subsequent development reached in the prostate.

Hormone injections

Male hormone. It has been shown earlier that the prostate and seminal vesicles of adults depend upon the testes to maintain their size and secretory differentiation. Injections of suitable dosages of male hormone into adult castrates prevent the appearance of all castration changes or repair them if they have been allowed to develop (Moore, Price and Gallagher, '30). Administration of male hormone to normal males produces testicular damage and decrease in testis weight (Moore and Price, '32).

The present study demonstrates that both glands continue to grow for a limited time after very early castrations. It was of interest to study the reaction of very young castrates and normals to male hormone administrations.

Table 3 presents the data from four out of nine litters treated with male hormone. Litter 1 will serve as an example of the effect of short time administration of male hormone on young castrates and normals. In a litter of five rats, three were castrated at 2 days. Two of these castrates and one normal were injected from the third to the eleventh day with 5 b.u. of male hormone daily. They were killed with a control normal and castrate on the twelfth day of life.

A comparison of the testes of the untreated normal, 501 and the male hormone injected normal, 502, brings out that male hormone injection has reduced the weight of the testes to one-half that of the untreated normal. Seminiferous tubules of the untreated normal (ten measurements) average

TABLE 3
Effects of injection of male hormone into young normal and castrate rats

LITTER	RAT	TREATMENT	AUTOPSY		TESTIS		SEMINAL VESICLE			PROSTATE	
			Age	Weight	Weight	Tubule diameter	Weight	Sec. gr.	Cell height	LA	Cell height
1 ¹	501	Normal control	days	gm.	gm.	μ	gm.		μ	+	μ
	503	Castrate control	12	22	0.0578	70.0	0.0034	—	14.0	+	21.0
	502	Normal + 5 b.u. male hormone	12	22			0.0008	—	14.7	—	
	504	3-11 days	12	23	0.0286	63.0	0.0054	—	14.0	+	21.7
	505	Castrate + 5 b.u. male hormone	12	24			0.0028	—	14.7	+	29.3
2 ¹		3-11 days	12	21			0.0024	—	15.4	+	20.3
	325	Normal control	12	21	0.0440	69.3		—	16.8	+	19.6
	328	Castrate control	12	21				—	16.1	—	
	323	Normal + 3-6 b.u. male hormone 1-11 days ³	12	24	0.0320	65.8		—	14.0	+	19.6
	895	Normal control + olive oil 6-14 days ⁴	15	28	0.0562	72.8	0.0038	—	15.4	+	21.0
3 ²	896	Castrate control + olive oil 6-14 days	15	27			0.0024	—	14.0	—	16.1
	893	Normal + 6 b.u. male hormone 6-14 days	15	30	0.0514	70.7	0.0156	+	21.0	+	21.7
	894	Castrate + 6 b.u. male hormone 6-14 days	15	32			0.0180	+	22.4	+	23.2

¹ Castrations performed at 2 days of age.

² Castrations performed at 5 days of age.

³ Three b.u. male hormone for 9 days; 6 b.u. for 2 days.

⁴ Olive oil extract of beef heart in amounts equivalent to the male hormone.

70 μ in diameter and those of the injected normal 63 μ . This difference is not large, but it has been found that in six out of seven litters treated with male hormone the normal testis tubules are larger than those of the male hormone injected normals; dosage in the seventh litter was 3 b.u. daily and the injection period short. This inhibiting effect usually is more marked after longer injection periods.

The seminal vesicle weight of the injected normal is greater than that of the normal control, and the glands of the injected castrates, 504 and 505, are three times that of the castrate control, 503, but are not up to the weight of the normal control. Microscopic study shows that the administration of male hormone for this period has had no detectable histological effect on seminal vesicle epithelium in either normal or castrates. The glands are all simple in structure and have low epithelium.

The prostate, however, gives a different result. Here male hormone has produced in the castrates, acini with lumina although the castrate control has solid acini. In addition, the injected castrates have prostates with high epithelium containing light areas that are as strongly developed as those in the untreated normal control or the injected normal. Acinous diameter has been definitely increased in the injected castrates and possibly increased in the injected normal although this may be only normal variation. The strength of the light areas in the injected normal is not increased.

Male hormone injections in this litter have reduced testicular weight by one-half and tubular diameters slightly, and have increased seminal vesicle size, and prostate acinous size in addition to developing acini and light areas precociously in castrates. Litter 2 substantiates the testis findings.

In litter 3 in which the dosage was 6 b.u. daily from 6 to 14 days, all of these effects were again produced except for the reduction in testis weight which was not so great. The differences are that seminal vesicle weights in the injected normal and injected castrate animal are five to six times greater than the normal control. Here the seminal vesicles of the injected castrate weigh more than those of the injected normal. With

this dosage the seminal vesicles of the injected animals now have high epithelium with a wide protoplasmic margin and small secretion granules precociously produced. In the prostate, light areas of both injected animals are stronger and the acinous diameter greater than in the untreated normal control.

In three other unlisted litters in which the injection periods continued to 18, 20, and 21 days of age, respectively, the earlier findings were extended in regard to testis inhibition. Not only were weight and tubular diameter decreased but the development of lumina in the tubules was inhibited. These develop in the normal testis tubules between 15 and 18 days. In normals injected with male hormone some lumina had developed at 18 days but not so many as in the normal controls and the difference was still more marked at 20 and 21 days. Degenerative changes in the germinal epithelium itself were not seen but inhibition was evident from these other findings.

In summary, the administration of male hormone in sufficient dosage has influenced the testes by reducing their size, weight, and tubular diameter, and by retarding the formation of lumina.

Male hormone affected the seminal vesicles of castrates by bringing them up to, or beyond normal weight and the seminal vesicle weights of injected normals were increased. The epithelium was increased in height, and secretion granules were produced at 15 days of age, that is, 3 weeks before normal differentiation takes place. In the castrate prostate, male hormone developed lumina in solid acini, brought out light areas before they would normally appear and increased acinous size, epithelial height and light area strength up to and beyond the state of the corresponding normal prostate. In the injected normals, the light areas, epithelial height and acinous diameter were all augmented beyond the usual normal state at that time. The injected castrates respond as well as, and in some cases better than, the injected normals.

Hebin (urinary) and phyone. Stimulation of the reproductive system of the rat by the administration of fresh

hypophyses, hypophysis extracts, raw pregnancy urine, and extracts of hypophysis-like substances from pregnancy urine is well known. Most of the work upon young male rats has been on those that were at least 2 to 3 weeks old. It seemed advisable to restudy the effects on young animals by the methods used in the preceding experiments. Urinary hebin (a gonad stimulating substance from pregnancy urine), phyone (the Wilson Laboratory growth promoting principle of the hypophysis extracted by the method of Van Dyke and Wallen-Lawrence, '30) and hypophysis implants were used on normals and on castrates.

Table 4 gives some of the results of these various experiments. In two litters (not given in table 4) injected 3 to 11 days it was demonstrated that in these young animals hebin has no effect upon castrate accessories and that phyone has no effect upon normal gonads and accessories or castrate accessories. In further litters, hebin was injected into normals, and phyone was tested further on normals with longer periods of injection and stronger dosage.

In litter 1 more prolonged phyone injection had no effect on the gonad or accessories of a normal but the effect of hebin on the young normal is well shown. This litter contained four rats of which one was given hebin from the third to the seventeenth day in the dosage of 1 r.u. per day for the first 2 days and 2 r.u. per day for the remainder of the period (13 days). The testes were about three times as heavy as those of the normal control and tubular diameters are increased. Interstitial tissue increase is marked. The normal testes have lumina as do also those of the hebin injected rat, but many tubules in the latter show a low germinal epithelium and vacuolated cytoplasm. This indicates an injury to the testes of young animals which was not observed (Moore and Price, '31) on slightly older rats. Both prostates and seminal vesicles gave a decided precocious response. This included increase in gross size and a secretory state indicating increased testis hormone influence. Secretion granules were produced at 18 days of age. Even stronger responses were

TABLE 4

Effects of hypophysectomy implants and injections of phyone and hebin in young normal and castrate rats

LITTER	RAT	TREATMENT	AUTOPSY		TESTIS		SEMINAL VESICLE			PROSTATE		
			Age days	Weight gm.	Weight gm.	Tubule diameter μ	Weight gm.	Sec. gr.	Cell height μ	LA	Cell height μ	Acinous diameter μ
1	477	Normal control	18	36	0.1552	92.4	0.0074	—	14.7	+	26.2	78.4
	472	Normal + phyone + hebin 3-7 days ²	8	10	0.0356	60.2	0.0032	—	5.9	—	17.5	44.8
	478	Normal + phyone 3-17 days	18	34	0.1118	95.9	0.0108	—	16.1	+	26.6	79.8
	479	Normal + 1-2 r.u. hebin 3-17 days ³	18	33	0.4598	121.1	0.0202	+	26.6	+	30.1	112.7
2	486	Normal control	22	52	0.2806	109.9	0.0092	—	12.6	+	21.7	94.5
	487	Normal + phyone 3-21 days ⁴	22	42	0.1722	108.5	0.0068	—	14.0	+	28.7	104.3
	488	Normal + phyone 3-21 days ⁴	22	46	0.2092	106.4	0.0086	—	14.0	+	28.0	105.7
	489	Normal + 1-2 r.u. hebin 3-21 days ³	22	45	0.7570	147.7	0.0694	+	21.0	+	35.0	114.8
3 ¹	924	Normal control	12	19	0.0408	60.2	0.0042	—	19.6	—	16.8	40.6
	925	Castrate control	12	26			0.0022	—	15.1	—	..	39.2
	926	Castrate control	12	19			0.0019	—	15.8	—	..	42.0
	927	Castrate + 14 young rat hy- pophyses 5-11 days ⁵	12	20			0.0010	—	15.1	—	..	33.6
	928	Normal + 14 young rat hy- pophyses 5-11 days ⁵	12	20	0.0930	72.8	0.0076	+	23.8	+	22.4	59.5
	929	Castrate + 7 adult rat hy- pophyses 5-11 days ⁶	12	18			0.0021	—	...	—	16.8	36.4
930		Normal + 7 adult rat hy- pophyses 5-11 days ⁶	12	20	0.0836	71.4	0.0074	+	21.0	+	23.8	60.9

¹ Castrations performed at 4 days of age.² One r.u. hebin + $\frac{1}{4}$ cc. phyone sample 36 in 1:6 dilution daily.³ One r.u. hebin for 2 days; two r.u. for the remainder of the injection period.⁴ One-eighth cubic centimeter twice daily phyone sample 36 in 1:6 dilution.⁵ Two hypophyses from male rats under 45 days of age implanted daily.⁶ One hypophysis from adult male rat implanted daily.

given in other animals (see litter 2). Hebin injections in young castrates (not listed in table 4) had no effect.

Phyone had no detectable effect on accessories of castrates or gonads or accessories of normals except that in stronger dosages in another litter there was an interstitial cell increase in the testis suggesting the presence of gonadotropic substance. Since phyone in strong dosages failed to exhibit any effect upon the accessory reproductive organs of castrates there is no indication that castrate prostate differentiation should be attributed to the growth promoting principle of the intact hypophysis.

Hypophysis implants

The response of the reproductive tract of the young male rat to fresh hypophyseal material has been well established by Smith and Engle ('27) and others. Moore and Price ('31) reported the effects of such implants on young males but the rats used were 20 days of age or over. To determine the degree of response of still younger males the hypophysis implantation technique was employed (see litter 3, table 4). It was considered a possibility that young and adult hypophyses might exert different effects, possibly directly, on early prostate differentiation.

In litter 3, four out of seven males were castrated at 4 days of age and three left normal. One normal and two castrates were kept as untreated controls and one normal and one castrate were given daily implantations of two hypophyses each from rats under 45 days old for 7 days (fifth to eleventh day). One normal and one castrate were given one adult hypophysis each, daily for the same period. The dosage was approximately 7 to 8 mg. daily in each of the two given young hypophyses, and approximately 10 to 12 mg. daily in each of the other two. The litter was killed on the twelfth day.

The castrates showed no effect upon the accessory organs from either young or adult hypophysis implants. The normals, on the other hand, gave a decided response. Testis weights were more than doubled and the tubular diameters

were increased. It can be noted that implantations of young hypophyses were fully as effective as adult hypophyses. The testis tubules are solid in the experimental animals (as is normal for this age) and there is no apparent effect on the germinal epithelium nor is there a visible increase in interstitial tissue.

The accessories show the stimulating effect of increase in male hormone. The normal control prostate has no light areas while those of both implanted normals have high epithelium and well-developed light areas. Mitoses are always numerous at this age but they seem especially conspicuous in the accessories of these implanted rats. The seminal vesicles were increased in weight and the high epithelium has a wide protoplasmic margin in which many small secretion granules with halos can be seen. This production of secretion granules at 12 days of age is a striking example of precocious development in glands that weigh less than 8 mg.

To summarize, the administration of fresh hypophysis material has no effect on the castrate prostate and seminal vesicles. In normals, it increases testis weight, tubular size and endocrine function. Both accessories are greatly stimulated secondarily through the testis, and secretion granules are produced in seminal vesicles of 12-day-old rats.

DISCUSSION

The question of the role of sex hormones in mammals during the entire course of development is one that has, as yet, no entirely satisfactory answer. The majority of secondary sex characters of adults have been shown to be dependent upon gonad hormones. However, how much of their origin, early growth, and differentiation is so conditioned is a more difficult problem.

Wiesner ('35) has put forth a new theory of sexual differentiation which he calls the 'monhormonic theory.' This is based upon a series of experiments involving castration of newborn male rats, spaying of newborn females and administration of male and female hormones, separately and in

combination. Some of these experimental results will be discussed in more detail below but a summary here will be sufficient to show the basis for his theory, which he extends to include embryonic as well as postnatal differentiation.

The experimental results of spaying or castrating newborn rats brought out the facts that, in the male, the growth and differentiation of the glans penis and seminal vesicles were inhibited by castration. The castration effects were corrected or nearly corrected by male hormone injections. In the female, spaying did not influence the development of the uterus, vagina and clitoris, nor did injections of female hormone into normal intact females accelerate growth or differentiation up to the third week of life although large doses (1 mg. total dosage) of oestrin resulted in some enlargement of the uterus and vagina. In older prepuberal females, female hormone is very effective as has been shown by many workers.

From this, Wiesner suggests that differentiation of the reproductive accessories is 'monhormonic.' That is, differentiation in the male including wolffian duct development and the impression of male characterization on the anlagen of the external genitalia depends on the secretion of the testis and cannot proceed without it. The müllerian ducts of males are thought to be destroyed by male hormone or because of the male constitution of their elements.

Differentiation in the female, on the other hand, does not depend upon any reaction between genital primordia and hormones but goes on independently (up to a certain stage, of course) and is dependent upon the genetic constitution of the somatic cells which may also cause involution of the wolffian system. Female differentiation is assumed to result from an anhormonic state both in the zygotic male and the zygotic female.

The present study of the embryology of the accessory reproductive glands of both male and female rats has shown that there is no sexually indifferent stage of the glands. Those of the male (especially the prostate) are very much larger than

those of littermate females even in the anlage stage. In addition, they grow much more rapidly and attain a striking development before birth. Is this due to the factor of maleness, i.e., is it genetic alone, or is there in addition the stimulating effect of male hormone from the testis to spur on growth as Wiesner suggests? This brings up the question of the time when male hormone is produced in the embryo.

Womack and Koch ('32 a) have proved the presence of male hormone in foetal bull testes by extraction methods. Benoit ('29) measured the combs of newly hatched Leghorn chicks and found that the combs of the males are significantly larger than those of the females. This difference at hatching indicates that the male comb has been growing under the influence of male hormone which has originated probably sometime toward the end of embryonic life according to Benoit.

Since male hormone (of the type produced in the adult) is present in the testes of fowls and calves it is possible that male hormone may be secreted in the foetal rat testis before birth. Wiesner supposes that it is produced early in embryonic development and may influence the development of the testis itself. He postulates a male hormone effect on determination of accessory organs as well as differentiation (see the discussion of the papillae of the glans penis below). If this is so, the great number of prostatic anlagen in the male as opposed to the single or paired primordia of the female homologue may be due at least in part to the presence of male hormone in the male. The female homologue—para-urethral glands—develop under anhormonic conditions and are carried up to a certain very low degree of development by some factor genetic or otherwise after which they degenerate in almost all female rats. Bartholin's gland (the female homologue of Cowper's gland) exhibits the same behavior. It is not clear what mechanism is operating here.

It has been stated that development of the reproductive accessory glands probably takes place in the presence of hormones (at least of the male) before birth. The influence of

male hormone from the testis very soon after birth has been demonstrated by Wiesner and in this paper. Castration of the rat within 12 hours of birth, in his study, inhibited the growth and differentiation of the glans penis and the seminal vesicles. Castration from 2 to 6 days of age has been shown in this work to affect prostate and seminal vesicle growth and differentiation. Our results are in agreement with Wiesner's on the seminal vesicle and further discussion can be omitted.

Wiesner's study of the effect of castration on the glans penis (which is incompletely differentiated at birth) brought out that the growth rate is reduced, and the ultimate size smaller. Differentiation, however, is not completely inhibited and the results differ with different constituents. The main results on the glans penis are: 1) No anterior process is formed, 2) a shorter and thinner but ossified os priapi occurs, 3) cavernous tissue of the sinus is reduced, 4) the corpus cavernosum glandis is incompletely differentiated, 5) there is incomplete differentiation of the integument, less papillae are formed (second week of post natal life) and they do not develop. The effects of castration become noticeable within one week after the operation.

It must be noted that while differentiation is affected it is not stopped and papillae which normally develop days after birth do so to some degree after very early castration and this partial development occurs at the same time that normal development would occur. The number of papillae in the castrate is smaller than in the normal and Wiesner takes this as an indication that male hormone affects not only final development, but also formation of the rudiments of papillae. Castration does not prevent the occurrence of all papillary anlagen because the integument is already partially determined at birth. He states that the degree of determination attained at the time of birth by the various glans penis constituents varies greatly and does not completely correspond with the degree of differentiation reached at birth.

The prostate of the castrate rat resembles somewhat in its behavior the development and differentiation of the glans

penis. The gland increases in gross size, the solid acini develop lumina and become distended, and the epithelium becomes high and acquires pale light areas, which are characteristic of the normal adult gland. The castrates vary greatly in the degree of differentiation they attain but of eighteen animals castrated in the first week and killed between 17 and 35 days of age, all but two had prostates that showed at least a few pale light areas in moderately high epithelium.

High epithelium and presence of light areas in the prostate have been used in previous work as criteria of a functional gland, for adult castration causes the loss of light areas and the reduction of epithelial height. How can one account for the development of distended acini, moderate epithelium and light areas in the prostate after castration and the maintenance of all these to some degree for at least 2 weeks after their development? There are at least three possibilities: 1) removal of the young testes may not result in such rapid disappearance of the testis hormone as in the adult; 2) some unknown factor perhaps genetic or hypophyseal may act upon the glands up to puberty at which time they may become exclusively dependent upon male hormone alone; or 3) the glands may be determined by male hormone before the time of castration.

The appearance of the prostate glands of young castrates differs from the normal only in quantitative respects which suggests differences in hormonal concentration. The castrate glands are frequently similar in appearance to Hansen's ('30) descriptions of adult castrate glands in which only a few peripheral acini respond to injection of male hormone because the dosage is too low to affect the remainder. Hansen shows that the prostate reacts to exceedingly minute amounts of male hormone.

However, it is difficult to see how the testes of 1- to 6-day-old animals could leave a sufficient residue of male hormone in the blood stream for a long enough time after testis removal to develop the prostate. This could obtain only if the hormone concentration is below the renal threshold or the

renal threshold is higher than in adults. Womack and Koch ('32 b) have reported that no male hormone (as assayed by comb growth methods) was found in urine of boys under 10 years of age but the urine of adolescent boys yielded quantities of hormone of the same order as that of mature men and pregnant and non-pregnant women. Bühler ('33) states that no male hormone was found before puberty in boys, but between puberty and 40 years the yield was 2 b.u. per 24 hours; between 40 and 60 years, less than 1 b.u. and after 60, none. Failure to secure positive results on prepuberal boys and also on calves under 2 years of age, although Womack and Koch ('32 a) had found calf and foetal testis tissue richer in hormone content than adult or old bull testes, brought Koch to say "the negative results on prepubescent boys' and calves' urines suggest that either the hormone is not secreted so rapidly as in the adult, that it is destroyed or that the kidney-threshold is at a very different level." He believes the first suggestion is more logical. These researches are suggestive but unfortunately nothing definite is known of the kidney-threshold.

The problem of the possible amount of hormone circulating in the blood stream after castration can be approached by examining the data on the appearance of castration cells in the hypophyses of young and old castrates. Hohlweg and Dohrn ('31) performed castrations on rats 2 weeks of age and on adults, and found that castration cells began to appear in the hypophyses of both groups by 12 days after castration indicating that the hormone disappeared from the young castrate as rapidly as from the adult. However, there is direct evidence to show that even when castration cells are found in the hypophysis enough male hormone can still be present to keep the prostate in a normal state, for Nelson ('34) found in long-time cryptorchid rats that castration cells appear in the hypophysis and the seminal vesicle glands become castrate in appearance but the prostate remains normal.

This finding is related to the threshold differences between the prostate and seminal vesicle which have been evident from the beginning of work on these glands and have been well

demonstrated by Hansen ('30). The fact that in our study the prostate appears very near the normal adult state between 15 and 20 days of age while the seminal vesicle does not have secretion granules until 36 days may mean nothing more than that the hormone content increases in the animal until the threshold of the seminal vesicle is reached. The prostate threshold is so low that the gland reaches a functional state very early.

The first attempted explanation for development of the castrate prostate leaves much to be desired. The second suggestion—that some unknown factor or factors may bring the gland up to puberty, after which it is carried on by hormone—is very like Wiesner's hypothesis for the development of the uterus and vagina of the spayed female except that he limits the factors to the genetic constitution of the somatic cells (or some extra-cellular factors). In his researches on the female he found only five cases out of twenty-nine of impaired development of the uterus and vagina after spaying. These five impaired cases he considered as possibly explainable on the basis of injury to blood vessels at the time of operation. The other twenty-one cases were not considered to differ significantly from the normal and were presumably quite consistent.

In the prostates of the castrate males, however, there was great variation in the amount of differentiation attained and even the best differentiated glands were never like those of the control littermates. If some factor operates to bring the glands to puberty it must be a highly variable factor to account for the experimental findings. If such a factor is ordinarily operating in every young normal male (in addition to the male hormone effect present) one might reasonably expect to find more consistent results in the glands of castrates.

The third suggestion was that the prostate may be determined before castration by male hormone (as Wiesner suggests for the glans penis). This, of course, cannot be ruled out as a possibility but as was stated above there seems to have been greater variation in the differentiation of castrate

prostates than was found by Wiesner in the glans penis. If development and differentiation of the castrate prostate depend upon determination by male hormone there must be an extraordinary amount of variability in the male hormone content of young rats at birth and for a few days thereafter. Again, the anlagen of papillae in the integument of the glans penis appeared at the same time in castrates and normals while light areas (which attain a nearly normal state as opposed to the undeveloped state of the papillae in castrates) do not develop until 17 days in the castrate although the normal intact male has them at 12 days. This suggests a lagging and submaximal response to a low hormone concentration. However, it might be explained on the basis that after early determination (which is only partial) a further stimulation by male hormone is necessary to produce light areas at the normal time.

The three hypotheses that have been set forth are all somewhat unsatisfactory and further research must be done before any definite conclusions can be drawn. The validity of the suggestion that development and differentiation of the glands occurs because of small amounts of residual hormone in the male is now being investigated.

The effects upon gonads and accessories of male hormone, hebin, and phyone injections, hypophysis implants, and castration in older animals have been, in general, in accordance with the findings of workers in this and other laboratories.

The effects of male hormone injections gave evidence of decided damage to the testis but a stimulating effect on the accessories to the point of producing secretion granules in the seminal vesicles at 15 days of age. Male hormone as administered to castrates by Wiesner offset castration effects on the glans penis but had no effect on the seminal vesicles. This appeared to be because of low dosages.

Results of urinary hebin injections agreed with earlier work (Moore and Price, '31) in producing great increase of testis weight, interstitial cell number, and size of accessories. Secretion granules were produced at 18 days. The diameters

of testis tubules showed a more definite effect here for they were markedly increased. However, damage was produced in the germinal epithelium and this was not evident in previous work. The longer injection period and heavier dosage might account for these differences in results. Also, since the injections were begun much earlier a different capacity of the tissue for reaction may be a factor.

The young testis responds readily to administration of gonadotropic hormones. This is not the case in the young ovary for Wiesner ('32 b) reports that injection of highly purified gonadotropic substances in dosages sufficient to cause oestrus or even pseudopregnancy in rats that were 17 to 56 days old did not produce any perceptible reaction in the ovary when injections were begun before 2 days of age. Uterine differentiation which occurs during the first 14 days of life was not accelerated by oestrifying hormone. Reactivity to oestrifying hormone develops gradually.

Hypophysis implants produced the expected effects in the increase of testis weight, enlargement of tubules, and great stimulation of the accessories and produced secretion granules in seminal vesicles of 12-day-old animals.

SUMMARY AND CONCLUSIONS

Normal development

a. The prostate of the male rat begins its development in the foetus at $19\frac{1}{2}$ days post coitum. The anlagen are multiple solid buds of cells from the urogenital sinus. Differentiation of the ventral lobe as judged by the presence of light areas in the cells is attained by 12 days after birth, and by 20 days the prostate is almost indistinguishable from that of the normal adult.

b. The prostate homologue in the female embryo (para-urethral glands of Skene) begins its development at about 20 days.

c. Seminal vesicles of the male originate in the foetus at about 19 days as paired diverticula from the wolffian ducts.

Secretory differentiation of the gland occurs at 36 days after birth when secretion granules appear.

d. There is no homologue of the seminal vesicle in the female rat.

Effects of prepuberal castration

a. Castration when the rat was less than a week old partially inhibited the growth of the prostate but did not prevent the development of distended lumina, moderately high epithelium, and pale light areas (characterizing the normal secreting gland) which developed at 17 days. Growth and differentiation were maintained only until the castrates were between 30 and 40 days of age when regression and atrophy set in.

b. Castration when the animals were 30 days of age or older was followed by regression of the prostate.

c. Development and differentiation of the castrate prostate may possibly be due to 'residual' male hormone, to 'fixation' by male hormone before the time of castration or to some other factor or factors.

d. Prostate glands of animals castrated at 2 days of age and as adults did not differ materially in histological structure after long periods of castration.

e. Castration within the first week of life slowed but did not entirely stop the growth of seminal vesicles. Their ultimate weight, however, did not exceed 7 mg. No differentiation took place.

f. Seminal vesicles of animals castrated at 2 days of age were very much lighter in weight after long periods of castration than those of adult castrates, but their histology was approximately the same.

Effects of hormone administration in young immature males

a. Male hormone injections, 1) produced light areas precociously in the castrate prostate (at 12 days) and increased the strength of reaction of the prostate of the normal; 2) increased the weight of castrate and normal seminal vesicles and produced secretion granules precociously in both (at 15

days); 3) reduced the weight of the testes, inhibited tubular development and reduced tubular diameters.

b. Phyone injection, had no detectable effect upon castrate or normal body weight, gonad or accessory gland weights, or histology except that testes showed an indication of interstitial cell increase after long injection.

c. Hebin (urinary), 1) had no effect upon castrate prostates but enlarged prostates of normals and produced a heightened secretory state (by stimulation of the testis); 2) had no effect upon the castrate seminal vesicle but stimulated the production of secretion granules at 18 days of age in injected normals, and increased the weight of the gland; 3) greatly enlarged the testes, increased tubular diameters and amount of interstitial tissues, and stimulated endocrine function of the gland as judged by the accessories. Damage to germinal epithelium was produced after longer injections.

d. Hypophysis implants, 1) had no effect on castrate prostates but caused slightly precocious development of light areas in the normal; 2) had no effect on castrate seminal vesicles but increased the weight of the normal and produced secretion granules in animals 12 days of age; 3) doubled testis weights and enlarged the tubules without seemingly increasing interstitial tissue. The endocrine function was greatly stimulated.

LITERATURE CITED

- BENOIT, J. 1929 Le déterminisme des caractères sexuels secondaires du coq domestique. Etude physiologique et histophysiologique. Arch. de Zool. Exp. et Gen., T. 69, p. 217.
- BÜHLER, FRITZ 1933 Sexualhormonbefunde im Harn von Männern verschiedenen Alters. Zeit. Ges. Exp. Med., Bd. 86, S. 650.
- CALLOW, ROBERT K. AND RUTH DEANESLY 1935 Effects of androsterone and male hormone concentrates on the accessory reproductive organs of castrated rats, mice and guinea pigs. Biochem. J., vol. 29, p. 1424.
- COMMINS, W. D. 1932 The effect of castrations at various ages upon the weight of male albino rats. J. Exp. Zool., vol. 63, p. 573.
- DISSELHORST, R. 1904 Ausführungsapparat und Anhangsdrüsen der männlichen Geschlechtsorgane. Oppel, Lehrb. d. vergl. mikr. Anat. d. Wirbelt. Teil IV.

- HANSEN, IRA B. 1930 Rat seminal vesicles and prostate glands as quantitative indicators of testicular hormone. *Endocrinology*, vol. 17, p. 163.
- HARRIS, J., C. JACKSON, D. PATTERSON AND R. SCAMMON 1930 The measurement of man. (The University of Minnesota Press.)
- HOHLWEG, WALTER AND MAX DOHRN 1931 Beziehungen zwischen Hypophysenvorderlappen und Keimdrüsen. *Wiener Arch. für innere Med.*, Bd. 21, S. 337.
- MARX, LORE 1932 Zur Anatomie der Prostata weiblicher Ratten. *Zeit. Zellforsch. und mikr. Anat.*, Bd. 16, S. 48.
- MOORE, CARL R., WINIFRED HUGHES AND T. F. GALLAGHER 1930 Rat seminal-vesicle cytology as a testis-hormone indicator and the prevention of castration changes by testis extract injection. *Am. J. Anat.*, vol. 45, p. 109.
- MOORE, CARL R., DOROTHY PRICE AND T. F. GALLAGHER 1930 Rat-prostate cytology as a testis-hormone indicator and the prevention of castration changes by testis-extract injections. *Am. J. Anat.*, vol. 45, p. 71.
- MOORE, CARL R. AND DOROTHY PRICE 1931 Some effects of fresh pituitary homimplants and of gonad-stimulating substance from human pregnancy urine on the reproductive tract of the male rat. *Am. J. Physiol.*, vol. 99, p. 197.
- 1932 Gonad hormone functions and the reciprocal influence between gonads and hypophysis with its bearing on the problem of sex-hormone antagonism. *Am. J. Anat.*, vol. 50, p. 13.
- NELSON, WARREN O. 1934 A study of the anterior hypophyses and sex-accessory glands of castrated and experimental cyptorchid rats. *Anat. Rec.*, vol 58 (Suppl.), p. 30.
- PALLIN, GUSTAF 1901 Beiträge zur Anatomie und Embryologie der Prostata und der Samenblasen. *Arch. f. Anat.*, S. 135.
- RAUTHER, MAX 1903 Ueber den Genitalapparat einiger Nager und Insektivoren, insbesondere die akzessorischen Genitaldrüsen derselben. *Jen. Zeit. Naturw., N.F.*, Bd. 31, S. 377.
- RIETSCHEL, P. E. 1929 Die Genitalausführungsgänge der wiessen Maus. *Zeit. Zoöl.*, Bd. 135, S. 428.
- SMITH, P. N. AND E. T. ENGLE 1927 Experimental evidence regarding the role of the anterior pituitary in the development and regulation of the genital system. *Am. J. Anat.*, vol. 40, p. 159.
- STUTZMANN, JULIUS 1898 Die akzessorischen Geschlechtsdrüsen von *Mus decumanus* und ihre Entwicklung. *Zeit. Naturwiss.*, Bd. 71, S. 257.
- VAN DYKE, H. B. AND ZONJA WALLEN-LAWRENCE 1930 On the growth-promoting hormone of the pituitary body. *J. Pharm. and Exp. Therap.*, vol. 40, p. 413.
- VAN WAGENEN, GERTRUDE 1928 Some effects of early castration on the growth of the male rat. *Am. J. Phys.*, vol. 84, p. 461.
- WALKER, GEORGE 1910 A special function discovered in a glandular structure hitherto supposed to form a part of the prostate gland in rats and guinea pigs. *Johns Hopkins Hosp. Bull.*, vol. 21, p. 182.

- WIESNER, B. P. 1932 The development of reactivity to gonadotropic hormone. J. Phys., vol. 75, p. 39.
- 1935 The post-natal development of the genital organs in the albino rat. J. Obst. and Gyn. of the Brit. Emp., vol. 41, p. 867 and vol. 42, p. 8.
- WOMACK, E. B. AND F. C. KOCH 1932 a Studies on the extraction of the testicular hormone from tissues and on its quantitative distribution therein. Endocrinology, vol. 16, p. 267.
- 1932 b The testicular hormone content of human urine. Endocrinology, vol. 16, p. 273.

PLATE 1

EXPLANATION OF FIGURES

($\times 250$ before reduction)

- 6 Prostate of a 15-day-old male, castrated at 4 days of age. Note the lumina forming in the acini.
- 7 Prostate of the normal 15-day-old littermate to the castrate in figure 6. A few light areas can be seen in the cells.
- 8 Prostate of a 25-day-old male castrated at 2 days of age.
- 9 Prostate of the normal 25-day-old littermate to the castrate in figure 8.
- 10 Prostate of a 30-day-old male castrated at 2 days of age. Note the presence of both high and low epithelium.
- 11 Prostate of a 52-day-old male castrated at 2 days. Littermate to the male in figure 10.
- 12 Prostate of an adult castrate, castrated for 21 days.

